

Cytological Changes in Fenugreek (*Trigonella foenum-graecum* L.) under Water Deficit Stress Induced by PEG-6000

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Abstract: The present study was carried out to determine cytological changes in root tip meristem of *Trigonella foenum-graecum* L., under water deficit stress induced by PEG - 6000. The seeds were pretreated with different water potentials (-0.1, -0.5, -1.0 MPa) of PEG - 6000 for 10 hours at 25±1°C. Mitotic index of root tip meristem declined gradually with decreasing water potential. Pronounced changes were recorded in cell size and chromosomal structure. Simultaneously the enhancement of mitotic anomalies like C-metaphase, sticky chromosomes in metaphase, chromosomal bridge at anaphase, vagrant chromosomes and breakage in various degrees at prophase and metaphase were observed.

Keywords: *Trigonella foenum-graecum* L., PEG – 6000, water potential, mitotic index, mitotic abnormalities, C-metaphase

Abbreviations: PEG - Polyethylene Glycol, MPa – Megapascal, FW - fresh weight, DW - dry weight, ABA- Abscisic acid, DNA – Deoxyribonucleic acid, ROS - reactive oxidative oxygen species.

1. Introduction

The global warming changes the climatic condition in earth causing either water deficit or flood and water logging. Drought is a major worldwide problem to crop production. It causes adverse effects on plants at different levels of their structure and function leading to ultimate loss of yield potential. Various physiological and biochemical changes have been observed in many plant species under water stress and salinity. Growth is intimately associated with mitotic division of meristematic cells. The contribution of processes like cell division and cell expansion to plant growth and yield has been studied in some crop species under such conditions [1]. Plant meristems can be affected by changes in temperature, water, irradiance and other biotic or physicochemical environmental stresses [2] involving changes in cell division and elongation [3]. The sensitivity of cell division to water stress can be attributed to the inhibition of the necessary metabolic requirements for this event [4] or to the restriction of meristematic cells from enlargement to reach the minimum size required for commencement of division [5],[3].

Trigonella foenum-graecum L., known as fenugreek, belonging to the family Fabaceae, is an annual herb cultivated in India and many other countries for various purposes such as medicinal uses, condiments in cooking, controlling insects in grain storages, perfume industries etc. Fenugreek is known to have various medicinal properties like antidiabetic, antiplasmodic, hypolipidemic, galactogauge, antibacterial, antihelmintic, anti-inflammatory, analgesic, anticancerous, chemo-protective and immunological activities [6],[7]. The plant is susceptible to water stress during the vegetative growth stage in contrast to other crops [8]. But the reports on mitotic division and chromosomal abnormalities in this crop under water stress are meager. Thus in the present investigation cytological

changes in fenugreek in response to PEG induced water stress were critically determined.

2. Material and Methods

Dried and healthy seeds of *Trigonella foenum-graecum* L. were surface sterilized with 0.1% mercuric chloride for 90 seconds followed by repeated washing in sterile double distilled water and soaked in different treatment solutions of polyethylene glycol (PEG)-6000 (-0.1, -0.5 and -1.0 MPa) and only sterile double distilled water as control (0 MPa) with three replications for 10 hours at 25±1°C. The pretreated seeds were set for germination in glass Petri dishes lined with double layers of filter paper [Whatman No. 1].

2.1 Sample Preparation for Cytological Study

Root tips were fixed in Carnoy's fixative I for 24 hours at 4°C, placed in 9:1 aceto-orcein (2%):1(N) hydrochloric acid, warmed gently for 5 seconds and kept for 2 hours. Stained root tips were squashed in 45% acetic acid on microscopic slides.

2.2 Microscopic Study

The prepared slides were observed under the light microscope (Leica DM 3000, Germany) for determining the following parameters:

- Mitotic index and abnormality index.
- Chromosomal structural abnormality.
- Cell size (length and width).

Mitotic index was estimated by the ratio of number of dividing cells and total number of cells scored. To determine the abnormality index the ratio of number of abnormal cells and total number of dividing cells was considered. Length

(µm) and width (µm) of the dividing cells were measured in different microscopic fields with ocular micrometer. Data were analysed statistically including mean value and standard error following [9].

3. Results

3.1 Mitotic Index and Abnormality Index

Mitotic index reflects the cell division frequency of an organ and is used to determine the root growth ratio as significant parameter. The decrease in mitotic index with increase in abnormality index and number of abnormal cells took place in root tips raised from seeds kept in increasing stressed conditions as compared to control set (Table 1). Treatment with PEG-6000 solution of -1.0 MPa water potential caused maximum number of abnormal cells with minimum mitotic index. The stage-wise abnormal cells of the root tips of different treated groups included chromosomal breakage at

prophase, C- metaphase, sticky chromosomes, vagrant chromosomes, disorientation of chromosomes and chromosomal breakage in metaphase, disturbed polarity and chromosomal breakage in anaphase (Table 1 and Figure 1). Although C-metaphase and sticky chromosomes were observed in the root tip meristems of all the treatments, the frequency of these abnormal cells was found to increase with increasing osmotic potential (Table 2).

3.2 Cell Length and Width

Table 1 shows that length of cells decreased gradually with the decreasing water potential. Though the increment in cell width was recorded at -0.1 MPa water potential, the width reduced gradually with lowering of water potential. Maximum reduction in cell size was noted in -1.0 MPa water potential.

Table 1: Changes in mitotic index, abnormality index and cell size (length and breadth) of root tip meristem of *Trigonella foenum-graecum* L. in response to PEG-6000 induced water deficit stress. Each value represents mean ± S.E. (n=3).

Water potential of PEG-6000 solution (MPa)	Mitotic index (%)	Abnormality index (%)	Cell length (µm)	Cell breadth (µm)
0	40.938** ± 0.79	0.583** ± 0.23	31.8** ± 0.13	22.36** ± 0.09
-0.1	30.623** ± 0.89	2.73** ± 0.33	30.16** ± 0.05	23.72** ± 0.43
-0.5	28.152** ± 0.70	5.521** ± 0.59	26.96** ± 1.2	21.11** ± 0.58
-1.0	25.271** ± 0.82	5.714** ± 0.08	24.21** ± 0.27	20.55** ± 0.12

Table 2: Number of different types of chromosomal abnormalities at various stages of mitosis in root tip cells of *Trigonella foenum-graecum* L. with different water potentials of PEG-6000

Water potential (MPa)	Number of cells	Number of different types of chromosomal abnormalities										
		Prophase		Metaphase					Anaphase			
		Breakage	C-Metaphase	Sticky chromosome	Vagrant chromosome	Disorientation	Breakage	Sticky Chromosome	Breakage	Disturbed polarity	Chromosomal Bridge	Laggard Chromosome
0	1258	-	2	-	1	-	-	-	-	-	-	-
-0.1	1316	-	3	5	-	2	-	-	-	-	-	-
-0.5	1158	1	4	7	3	1	-	1	-	1	-	-
-1.0	1419	2	5	9	1	1	1	1	1	-	2	1

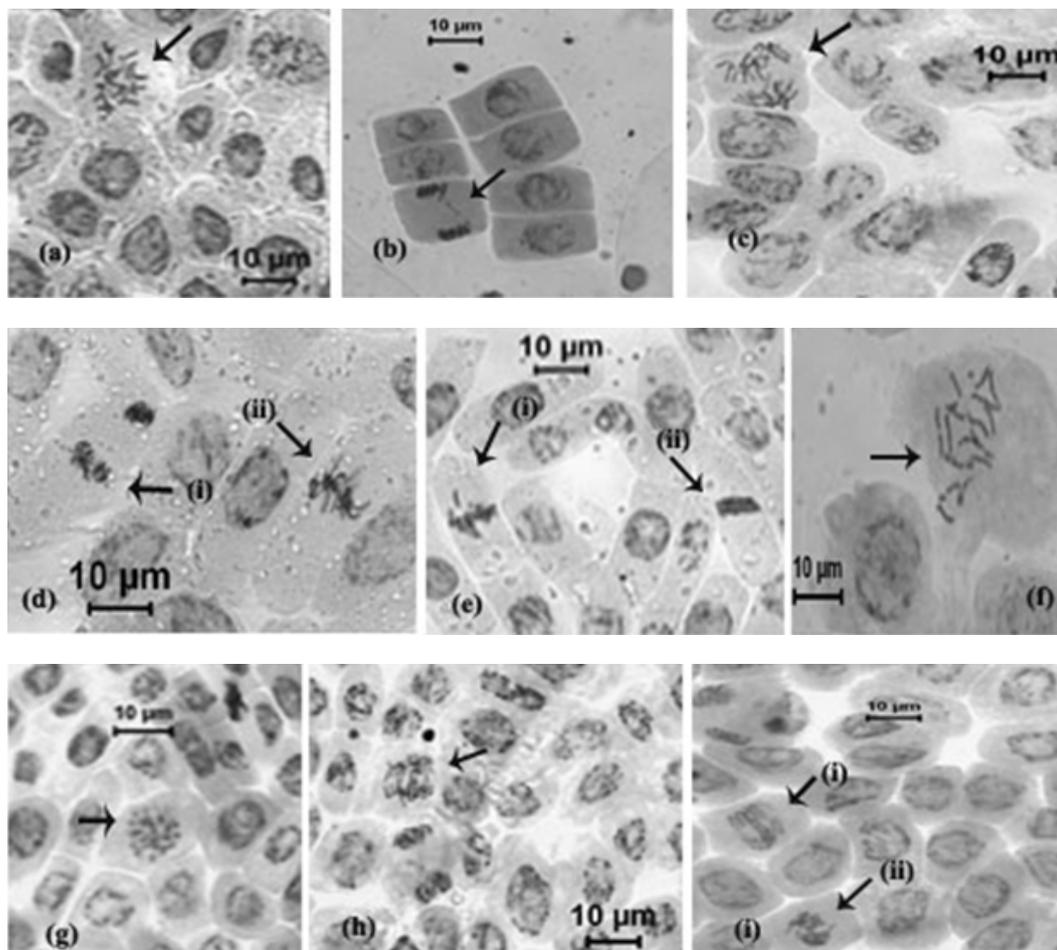


Figure 1: Photograph showing effects of water deficit stress induced by PEG – 6000 solutions of various water potentials on root tip meristem of *Trigonella foenum-graecum* L.

(a) C-metaphase (-0.1 MPa); (b) Laggard at anaphase (-1.0 MPa); (c) Disturbed polarity at anaphase (-0.5 MPa); (d)-(i) Sticky chromosome at anaphase (-0.5 MPa); (d)-(ii) Sticky chromosomes at metaphase (-0.5 MPa); e-(i) Vagrant chromosome at metaphase (-0.5 MPa); e-(ii) Sticky chromosomes at metaphase (-0.5 MPa); (f) C-metaphase with chromosomal breakage (-1.0 MPa); (g) Breakage at prophase (-1.0 MPa); (h) Chromosomal breakage at anaphase (-1.0 MPa); (i)-(i) Multiple bridge at anaphase (-1.0 MPa); (i)-(ii) Sticky bridge at late anaphase (-1.0 MPa);

4. Discussion

The water stress caused a mitodepressive effect on root tip cells of *Trigonella foenum-graecum* L. (Table 1). There are reports of rapid decrease in mitotic activity in roots after imposition of water stress in *Vicia faba* [3] and pea [10]. A similar response was found in soybean hypocotyl [11]. Similar types of cytological anomalies during meiosis were reported by Singh [12] in barley under water stress condition. In the life cycle of a plant a highly dehydration sensitive developmental phase comes immediately after germination [13], where many chromatin regulators act to trigger water stress-dependent growth arrest, which resembles the growth arrest during late embryogenesis in seed development [14]. Schuppler et al. [15] pointed out that water stress had induced a signal that increased phosphorylation of tyrosine at the active site of Cdc-2 kinase resulting accumulation of the inactive form of the enzyme

and consequently the progression into mitosis was inhibited. Some workers suggest that osmotic stress and regulation of cell cycle share common signaling pathways which can be influenced by ABA [16]. The present study showed different types of mitotic abnormalities in drought stressed root tip meristem of *Trigonella*. The pronounced effect spindle failure which caused C-metaphase, disorientation of chromosomes, disturbed polarity, laggard and vagrant chromosomes during cell division in root meristem.

It seems that the lower activity of Cdc-2 kinase due to drought stress may have caused impairment of spindle activity, because some workers suggest that at mitosis higher activity of this enzyme causes the structural changes of nuclear envelope disassembly, chromosome condensation and mitotic spindle assembly in yeast and animals [17-18]. Stickiness is a sign of toxic influence on the chromosomes probably leading to cell death [19]. Partial dissociation of nucleoproteins and alteration in the pattern of organization of chromosomes [20] or due to disturbances in cytochemically balanced reactions cause stickiness of chromosomes [21], which might have occurred in *T. foenum-graecum* L. due to water stress in cells. It may be possible that water deficiency may have caused some kind of gene mutation leading to incorrect coding of some non-histone proteins involved in chromosome organization. These proteins lead to stickiness. The chromosomal breakage indicates clastogenic effects. A secondary stress like oxidative stress in the condition of hyperosmolarity

develops due to higher amount of reactive oxygen species (ROS). ROS can have damaging effect on cellular structures and macromolecules especially DNA inducing numerous lesions that cause deletions, mutations and other lethal genetic effects [22].

The observed reduction in length and width of the cells (Table 1) was due to dehydration mediated cell shrinkage resulting consequent reduction in cellular volume [23].

5. Conclusion

The study provides the information that water deficit stress, given at the early stage, has an adverse impact on the fenugreek plant at adult stage, which may affect its genetic purity as well as yield potentiality by anomalous cytological behaviour.

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